

## Is the Coelomic Plasma of *Phascolosoma arcuatum* (Sipuncula) Hyperosmotic and Hypoionic in Chloride to the External Environment?

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**ABSTRACT**—Since *Phascolosoma arcuatum* lives in the mud of the mangrove swamp, which is subjected to extremely variable salinities, the present studies were undertaken to examine if this sipunculid could, to a certain extent, regulate ions and/or volume. When acclimated to 30‰ or 100‰ seawater (SW), *P. arcuatum* maintained its coelomic plasma (CP) slightly, but significantly, hyperosmotic to the external medium. Maintenance of a slightly hyperosmotic coelomic fluid would favor a constant, yet small, osmotic water influx which would compensate for urine production and any other process by which water is lost. The  $\text{Cl}^-$  concentration in the CP of this sipunculid was always lower than that of the external medium. The difference in the  $\text{Cl}^-$  concentration between the CP and the external medium in specimens acclimated to 100‰ SW was greater than that in specimens acclimated to 30‰ SW. Specimens acclimated to 100‰ SW also contained significantly greater concentrations of inorganic phosphate, organic phosphates and water-soluble proteins in the CP than those acclimated to 30‰ SW. The hypoionic  $\text{Cl}^-$  condition in the CP of this worm might be essential for the accommodation of negatively charged organic osmolytes in the CP without violating electroneutrality. Hence, it was concluded that *P. arcuatum* could regulate to a certain extent its ionic compositions in the CP and maintain the CP hyperosmotic to the external medium.

### INTRODUCTION

The question of whether sipunculids possess the ability to regulate body volume, especially in an environment of changed salinities, has been a matter of some controversy [12]. There have been studies on perhaps ten species but most of these reports are inconclusive. Some of these reports provide no data but just state that the worms do (or do not) regulate volume after a salinity transfer [13]. No species is known from areas with markedly low salinities. The only possible exception is the species *Phascolosoma arcuatum*, which was referred to as *Physcosoma lucro* by Harms and Dragendorff [6] or *Phascolosoma lucro* by Oglesby [13, 14] as reviewed by Rice and Stephens [21] and Stephens and Edmonds [24]. The high mangrove swamp habitat of this species, subjected to extremely variable salinities which is an atypical situation for a sipunculid, suggests that it may be able to regulate ions and/or volume.

The consistently hyperosmotic coelomic fluid of *P. arcuatum* [6] has been regarded as the result of active osmoregulation [5, 8]. However, Oglesby [12] suggested that such observations might have been caused by progressive desiccation in its semi-terrestrial habitat. On the other hand, Green and Dunn [4] claimed that the CP of this sipunculid kept for up to 64 hr in the laboratory in 40–100‰ artificial SW were uniformly isosmotic to the external media. They further reported that *P. arcuatum* showed no indication of volume recovery after a hypoosmotic or hyperosmotic salinity transfer. To date, whether *P. arcuatum* can main-

tain a hyperosmotic coelomic fluid to its external environment is still disputable, though it is apparent that few, if any, invertebrates are isosmotic with their environment [19]. Furthermore, although reports on the sipunculids, *Themiste dyscritum* [7], *T. signifer* [9] and *Phascolopsis gouldi* [23], confirm the  $\text{Cl}^-$  concentration of the CP to be hypoionic to that of the external medium, Green and Dunn [4] concluded that *P. arcuatum* was uniformly isoionic in terms of  $\text{Cl}^-$  to the external artificial SW. Hence, the present studies were undertaken to determine if the CP of *P. arcuatum* was hyperosmotic and hypoionic in  $\text{Cl}^-$  to its external medium. In addition, the concentrations of  $\text{Na}^+$ ,  $\text{HCO}_3^-$ , inorganic phosphate ( $\text{P}_i$ ), organic phosphate ( $\text{P}_o$ ) and water-soluble proteins in the CP were also determined to reveal the possible contribution of organic molecules to the maintenance of electroneutrality in, and to the total osmotic concentration of, the coelomic fluid of *P. arcuatum*.

### MATERIALS AND METHODS

#### Collection and maintenance of specimens

*P. arcuatum* were collected from the mud-flats of the mangrove swamp at Mandai, Singapore, and maintained at 25°C in the laboratory in aquaria with aerated 50‰ (15‰ salinity) SW. The worms were not fed. Experiments were performed after 10 days of acclimatization of the worms to laboratory conditions.

#### Determination of weight after transfer from 50‰ SW to 30‰ or to 100‰ SW

Individual worms (2.5–3.5 g) were gently blotted dry with moist filter paper, weighed and put in plastic cups containing 500 ml of 30‰ (9‰ salinity) or 100‰ (30‰ salinity) SW. Weight was measured to the nearest milligram with a Shimadzu Libror EB-280M balance.

Half of the water in the plastic cup was changed daily. At time intervals of 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 60 hr and 72 hr, the worm was carefully removed from the cup, blotted dry with moist filter paper and weighed.

#### Determination of water content in the tissues

In a separate experiment, worms were acclimated to either 30% or 100% SW for 48 hr. After 48 hr, the worm was sampled individually and an incision was made on the body wall to drain the coelomic fluid. The worm body was dissected open, gently blotted dry with moist filter paper, weighed and dried in the oven at 95°C to constant weight. The water content was calculated as the difference between wet weight and dry weight of the sample.

#### Analyses of CP and the external medium

The worms in groups of 15 were acclimated to 10 l of aerated 30% or 100% SW for 48 hr. After acclimation, a sample of the external medium to which the worms were acclimated were collected in ice-cold test-tubes and various assays were performed on them within 3 hr. The worms were quickly blotted dry. A small incision was made on the body wall, from which the coelomic fluid was drained into ice-cold test-tubes. Coelomic fluid was centrifuged at  $1,000 \times g$  at 4°C for 10 min to obtain the CP. The osmolalities ( $\text{mosmol} \cdot \text{kg}^{-1}$ ) of the CP and SW samples were determined by a Wescor 5500 Vapour Pressure Osmometer (Wescor, UT, USA).  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations were determined using a Corning 410C Flame Photometer and Corning 925 Chloride Analyzer, respectively.  $\text{HCO}_3^-$  concentration was determined by a Corning 965 Carbon Dioxide Analyzer.  $\text{P}_i$  was analyzed using a Tecator Aquatec System equipped with a Phosphate Cassette according to the Tecator Application Note ASN 146-05/90. Total phosphate ( $\text{P}_T$ ) and protein analyses were performed on the CP of *P. arcuatum* only.  $\text{P}_T$  was assayed by the Tecator Aquatec System following the Application Note ASN 147-05/90.  $\text{P}_o$  concentration was calculated as the difference between the concentrations of  $\text{P}_T$  and  $\text{P}_i$ . Water-soluble protein present in the CP was determined by the method of Bradford [2]. Bovine gamma globulin dissolved in 25% glycerol was used as a standard for comparison.

#### Statistics

Results were presented as means  $\pm$  standard error (SE). Student's *t* test was used to compare differences between means. Differences with  $P < 0.05$  were regarded as statistically significant.

## RESULTS

After being transferred from 50% SW to 30% SW, *P. arcuatum* rapidly gained weight (14% after the first 6 hr of exposure), presumably due to an osmotic influx of water (Fig. 1). Conversely, specimens transferred from 50% SW to 100% SW for 6 hr lost 31% of their initial body weight (Fig. 1). This sipunculid was able to recover its volume partially from the water gained and lost in 30% SW and 100% SW, respectively, and reached a steady state after 48 hr. The water content in the body tissues increased from  $72.7 \pm 1.1\%$  in 50% SW to  $77.4 \pm 0.4\%$  after 48 hr in 30% SW. The water content in the body tissues of worms acclimated to 100% SW for 48 hr was  $68.0 \pm 0.3\%$ , a loss of 4.7% compared to that of the worms kept in 50% SW. Since *P. arcuatum* appeared to reach a steady state with its environment after 48 hr, all subsequent experiments were performed with worms acclimated to 30% or 100% SW for this period of time unless

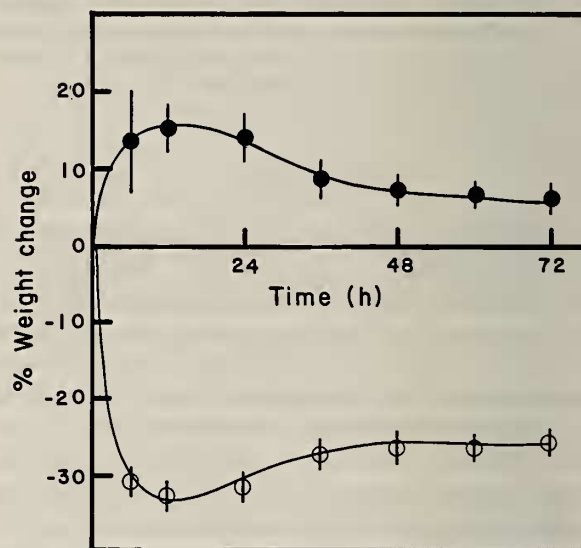


FIG. 1. A time course study on the percentage of weight change in *P. arcuatum* after it has been transferred from 50% SW to 30% SW (●) or 100% SW (○) for three days. Each point represents the average weight of 7 individuals. Vertical bars show standard errors.

TABLE 1. Osmolality ( $\text{mosmol} \cdot \text{kg}^{-1}$ ) of and  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$  and  $\text{P}_i$  (inorganic phosphate) concentrations (mM) in the external medium (EM) in which specimens had been kept for 48 hr and the coelomic plasma (CP) of *P. arcuatum* exposed for 48 hr to 30% or 100% SW

SW	Sample	Osmolality	$\text{Na}^+$	$\text{Cl}^-$	$\text{HCO}_3^-$	$\text{P}_i$
30%	EM	$310.9 \pm 2.0$ (7)	$136.5 \pm 0.6$ (6)	$180.3 \pm 1.3$ (7)	$1.46 \pm 0.06$ (7)	$0.0029 \pm 0.0001$ (7)
	CP	$316.9 \pm 1.3$ (7) <sup>1</sup>	$136.0 \pm 0.5$ (6)	$174.1 \pm 0.9$ (7) <sup>1</sup>	$4.22 \pm 0.22$ (7) <sup>1</sup>	$0.055 \pm 0.007$ (7) <sup>1</sup>
100%	EM	$1022.3 \pm 1.9$ (7)	$470.6 \pm 3.9$ (7)	$567.1 \pm 1.1$ (7)	$1.51 \pm 0.03$ (7)	$0.0097 \pm 0.0010$ (7)
	CP	$1034.1 \pm 2.0$ (7) <sup>1</sup>	$479.6 \pm 2.7$ (7)	$552.6 \pm 1.6$ (7) <sup>1</sup>	$3.23 \pm 0.16$ (7) <sup>1</sup>	$0.69 \pm 0.34$ (7) <sup>1</sup>

Data represent means  $\pm$  SE with the number of determinations in parenthesis

<sup>1</sup> significantly different from the corresponding EM value ( $P < 0.05$ ).



stated otherwise. No significant change in weight was observed for specimens transferred from 50% SW to 50% SW for 48 hr.

The osmolalities of the CP of worms acclimated to 30% or 100% SW were slightly, but significantly, higher than those of the respective external media (Table 1). Similar results were obtained when the worms were acclimated to 30% or 100% SW for seven days. The concentration of  $\text{Na}^+$  in the CP of these specimens was not significantly different from that of the respective external media, but the  $\text{Cl}^-$  concentration in the CP was always significantly lower than that of the external medium (Table 1). In contrast, the  $\text{HCO}_3^-$  and  $\text{P}_i$  concentrations in the CP of specimens acclimated to 30% or 100% SW were significantly higher than the respective values in the external media.

The  $\text{P}_T$  ( $1.19 \pm 0.22$  mM,  $n=7$ ) and  $\text{P}_o$  ( $0.50 \pm 0.09$  mM,  $n=7$ ) concentrations in the CP of *P. arcuatum* acclimated to 100% SW were significantly greater than those ( $0.109 \pm 0.016$  mM,  $n=7$  and  $0.055 \pm 0.007$  mM,  $n=7$ , respectively) of the specimens acclimated to 30% SW. The water-soluble protein concentration in the CP of the former ( $2.37 \pm 0.24$  mg·ml $^{-1}$ ,  $n=5$ ) was also significantly greater than that of the latter ( $0.86 \pm 0.18$  mg·ml $^{-1}$ ,  $n=5$ ). Such differences in the  $\text{P}_T$ ,  $\text{P}_o$  and water-soluble protein concentrations could not be explained by the increase and decrease in water content of the CP in 30% and 100% SW, respectively. After recalculation to eliminate the respective dilution and concentration effects, the water-soluble protein concentrations of worms in 30% ( $0.93 \pm 0.19$  mg·ml $^{-1}$ ) and 100% SW ( $1.71 \pm 0.18$  mg·ml $^{-1}$ ) were still significantly different from each other.

## DISCUSSION

Contrary to the report of Green and Dunn [4], *P. arcuatum* lost some of the water gained initially in 30% SW and reached a steady state of 6.8% greater than the initial weight by the 48th hr. Similarly, specimens were able to regain some of the water lost initially after being transferred from 50% SW to 100% SW. Our results also contradict those of Green and Dunn [4] by confirming *P. arcuatum* to be capable of maintaining its CP slightly, but significantly, hyperosmotic to its external medium. Maintenance of a slightly hyperosmotic coelomic fluid would favor a constant, yet small, osmotic water influx which could compensate for urine production and any other processes by which water is lost [16]. Thus, the majority of invertebrates [15, 18, 20] and some vertebrates (hagfish, shark and rays, the coelacanth, and the crab eating frog) [10] are always slightly hyperosmotic although they are osmoconformers.

In addition, contrary to Green and Dunn's [4] claim, our results verify that the  $\text{Cl}^-$  concentration in the CP of *P. arcuatum* was always significantly lower than that of the external medium, be it 30% or 100% SW. In this regard, it is important to notice that no standard deviation or SE was presented for the data on the external media in the report of Green and Dunn [4], and the comparison of the  $\text{Cl}^-$  concen-

tration in the CP to that in the external medium in their studies was apparently subjective.

The cause of the hypoionic  $\text{Cl}^-$  condition in the CP of sipunculids is still uncertain [13]. One hypothesis is that concentrations of inorganic anions, such as  $\text{HCO}_3^-$  or  $\text{SO}_4^{2-}$ , may be considerably higher in the coelomic fluid than in the external medium. However, measured  $\text{SO}_4^{2-}$  concentrations in sipunculid coelomic fluids are lower than those in the external SW [1, 22]. Our results indicate that  $\text{HCO}_3^-$  and  $\text{P}_i$ , though present in significantly greater concentrations in the CP than in the external media, could only partially account for the difference in  $\text{Cl}^-$  concentration between these two samples.

Gross [5] suggested that osmotically active particles, of an unspecified chemical nature, could be exchanged between the coelomic fluid and the tissues of sipunculids, altering the osmotic concentration and, therefore, composition of the coelomic fluid during certain condition of osmotic stress. If the particles involved were negatively charged at physiological pH, regulating the  $\text{Cl}^-$  concentration in the CP of *P. arcuatum* to be consistently lower than that of the ambient water would be essential to maintaining electroneutrality of the ions present. Indeed, *P. arcuatum* is capable of using free amino acids (FAAs) to regulate cell volume [17]. After a downward transfer from 50% to 30% SW, there is a release of FAAs from the body tissues into the CP. When being confronted with an upward transfer from 50% to 100% SW, *P. arcuatum* exhibits a small uptake of FAAs from the CP by the body tissues during the initial phase of the transfer [17]. However, most FAAs, being zwitterions, do not carry any net charge at physiological pH. Hence, some other organic osmolytes must be involved in maintaining electroneutrality in the CP of this sipunculid.

$\text{P}_o$  and water-soluble proteins are normally negatively charged at pH 7.42 of the coelomic fluid of *P. arcuatum* [11]. Our results indicate that the CP of specimens acclimated to 100% SW had significantly greater concentrations of  $\text{P}_o$  and water-soluble proteins than that of specimens acclimated to 30% SW. In agreement with such an observation, the difference in  $\text{Cl}^-$  concentration between the CP and the ambient medium in specimens acclimated to 100% SW was greater than that in worms acclimated to 30% SW. Since worms fasted for 10 days were used through out the experiment, it is reasonable to assume that this increase in protein concentration reflects the degradation of some proteins in the tissues and the release of smaller water-soluble units into the CP. Indeed, Peng *et al.* [17] reported that the significant increase in the total FAA content in the tissues of the worms acclimated to 100% SW was mainly due to an increase in protein catabolism. Chew *et al.* [3] further demonstrated that increased protein catabolism occurred in the internal organs, but not in the body wall of *P. arcuatum* in such a condition. Some of the water-soluble proteins could have been released from the internal organs to the CP to be transported to the tissues of the body wall. In addition, the release of proteins into the CP of worms acclimated to 100%

SW might be essential to the maintenance of hyperosmoticity, since some of the FAAs in the CP were absorbed by the body tissues [17].

Hence, we concluded that *P. arcuatum* could regulate to some extent its volume and ionic composition as for its CP. This is in agreement with the results of Harms and Dragendorff [6] and Gross [5].

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